

environment. The sample may be treated with sodium dodecyl sulfate containing glycine hydrochloride and then plated onto a growing medium. After an incubation period, an inspection of one or more bacterial growth colonies on the growth medium may determine the presence of *Mycobacterium* in the environment.

**[0023]** In one embodiment, the growing medium may be an agar based growth medium comprising agar, one or more amino acid and nitrogenous supplementation elements, one or more trace elements and vitamins, one or more carbon sources, one or more neutralizing agents and crystal violet for differentiating non-*Mycobacterium* from *Mycobacterium*. The crystal violet may be provided in an amount in excess of 0.5 µg/ml.

**[0024]** In one aspect, there is provided a growth medium for the growth of *Mycobacterium*. The growth medium may include agar, one or more amino acid and nitrogenous supplementation elements, one or more trace elements and vitamins, one or more carbon sources, one or more neutralizing agents and crystal violet for differentiating non-*Mycobacterium* from *Mycobacterium*. The crystal violet may be provided in an amount in excess of 0.5 µg/ml.

**[0025]** The above description sets forth, rather broadly, a summary of one embodiment of the present invention so that the detailed description that follows may be better understood and contributions of the present invention to the art may be better appreciated. Some of the embodiments of the present invention may not include all of the features or characteristics listed in the above summary. There are, of course, additional features of the invention that will be described below and will form the subject matter of claims. In this respect, before explaining at least one preferred embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of the construction and to the arrangement of the components set forth in the following description or as illustrated in the drawings. The invention is capable of other embodiments and of being practiced and carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein are for the purpose of description and should not be regarded as limiting.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0026]** FIG. 1 substantially depicts a comparison of a limited nutrient growth media and MYChrOme media on which *Mycobacterium* have been grown;

**[0027]** FIG. 2 substantially depicts a series of growth plates of different growth media and pretreatment on which *Mycobacterium* have been grown

**[0028]** FIG. 3 substantially depicts a flowchart of a method for detecting presence of *Mycobacterium* in an environment; and

**[0029]** FIG. 4 substantially depicts a flowchart of an alternative method for detecting presence of *Mycobacterium* in an environment.

#### DESCRIPTION OF CERTAIN EMBODIMENTS OF THE PRESENT INVENTION

**[0030]** In the following detailed description of the preferred embodiments, reference is made to the accompanying drawings, which form a part of this application. The drawings show, by way of illustration, specific embodiments in which the invention may be practiced. It is to be understood that other embodiments may be utilized and structural changes may be made without departing from the scope of the present invention.

**[0031]** To aid the detection and differentiation of *Mycobacterium* colonies, there is provided a growth media for-

mulation that is able to target strains of *Mycobacterium*, in particular non-tuberculosis *Mycobacterium* (NTM). For ease of reference throughout the remainder of this specification, the growing media will be referred to by the present Applicant's proprietary term MYChrOme™. The growth media formulation for MYChrOme includes a limited nutrient media containing an unusually high amount of crystal violet. Examples of the limited nutrient media include R2A, Middlebrook agar, and Plate Count Agar. Components of the nutrient media may include combinations of proteose peptone, casamino acids, yeast extract, dextrose, soluble starch, dipotassium phosphate, magnesium sulfate, sodium pyruvate, and agar. In one embodiment, the crystal violet may be added to the media in an amount of 0.5-5.0 µg/ml. In one embodiment, the concentration of crystal violet in the growing medium is at least 1.0 µg/ml. In one embodiment, the concentration of crystal violet in the growing medium is at least 1.5 µg/ml. In one embodiment, the concentration of crystal violet in the growing medium is at least 2.0 µg/ml.

**[0032]** In one specific example, the growth medium may contain an agar based compound. The growth medium may include one or more amino acid and nitrogenous supplementation elements, one or more trace elements and vitamins, one or more carbon sources, one or more neutralizing agents and crystal violet for differentiating non-*Mycobacterium* from *Mycobacterium*.

**[0033]** In one embodiment, the crystal violet may be provided in an amount of 0.5-5 µg/ml.

**[0034]** In one embodiment, the growth medium may include 0.25-1.5 g/L of proteose peptone and 0.25-1.5 g/L casamino acids to provide necessary amino acids and nitrogenous supplementation, 0.25-1.5 g/L yeast extract to boost growth and as a supply of trace elements and vitamins, 0.25-1.5 g/L dextrose as a carbon source, 0.25-1.5 g/L soluble starch as a neutralizing agent.

**[0035]** In addition, the growth medium may include 0.1-1.0 g/L sodium pyruvate to aid the growth of stressed microbes, 0.01-1.0 g/L magnesium sulfate and 0.1-1.0 g/L dipotassium phosphate to maintain osmotic equilibrium.

**[0036]** Agar may be provided as the solidifying agent in an amount of 10-20 g/L.

**[0037]** Bacteria-containing samples can be inoculated onto this growth media. In a liquid formulation the purple-pigmented media will turn colorless in the presence of *Mycobacterium*. In a solid formulation the media causes *Mycobacterium* to grow white colonies (or retain their original pigment) while most other bacteria grow purple colonies, allowing for rapid identification of *Mycobacterium*, especially in samples that may be heavily contaminated with competing microbiota. That *Mycobacterium* can survive with such high concentrations of crystal violet was unforeseen and unexpected. Furthermore, it was unforeseen that most other bacteria tested could not metabolize the crystal violet.

**[0038]** To further facilitate the identification of the *Mycobacterium*, a sample that has been obtained from the environment and filter concentrated can be treated, prior to plating, with a compound that is selective for *Mycobacterium*. In one embodiment, the treatment compound comprises a final concentration of 1-5 mM glycine hydrochloride and 0.1%-1.0% sodium dodecyl sulfate (SDS). This compound, which will be referred to throughout this specification by the present Applicant's proprietary term MYCON™, has surprisingly been found to inhibit the growth of all bacteria and fungus tested thus far other than *Mycobacterium*. MYCON may be added to the filtered concentrate prior to plating and left for 5 minutes at room temperature. The